

DOCTORAL DISSERTATION SUMMARY

The doctoral thesis, entitled “*Wine industry originated resources with possibilities of valorification in the animal nutrition*” has a two-part structure: the bibliographical part and the own research part and it comprises 8 chapters, to which the general conclusions, the recommendations and the studied bibliography were added; the thesis has 277 pages, 50 tables, 144 illustrations and 350 bibliographical titles.

Part I. – **The present state of knowledge** consists of two chapters, relevant for the proposed topic: CHAPTER I. *Attainment and nutritional evaluation of the wine industry by-products, exploitable in animal nutrition* and CHAPTER II. *The use of some wine industry by-products in animal nutrition*.

In the second part of the thesis, **Own research**, starting from the general purpose of evaluating the chemical composition and establishing a preservation method for certain wine industry by-products, together with their use in the nutrition of certain poultry categories (broiler chickens and laying hens), I have particularized the research previously carried out, in order to obtain results with scientific value, based on which an original behaviour can be reformulated with the purpose of a better use of the winery by-products in the poultry nutrition.

In order to achieve the intended purpose, the research was carried out following **four main objectives**. **First objective was to establish a grape pomace preservation procedure, for an improved exploitation in the poultry nutrition**. The grape pomace preservation procedure was conducted taking into account the effects the drying temperature has on the amount of polyphenols contained by the grape pomace.

The second objective regarded **the determination and the comparative evaluation of the chemical content of grape pomace and vegetable fractions – seeds, skins and stalks** from several grape varieties obtained in different years, aiming to an optimal use in poultry nutrition. The grape pomace samples, gathered from three wine-growing centres: Huși vineyard, Adamachi farm of Iași, Center of Research and Development for Viniculture and Winemaking of Iași (SCVV), were brought to the biobase of the Faculty of Animal Breeding from USAMV Iași for drying and grape pomace fractions separation (seeds, skins, stalks). The chemical and nutritional description of these winery by-products was performed in the Faculty of Animal Breeding’s Fodder Quality Control Laboratory from the USAMV Iași and at the Poultry Research Unit (URA), within INRA in France.

The third objective was to establish the effect grape seeds, whole grape pomace and aqueous polyphenols extract from the grape pomace included in the broiler chicken's nutrition has on their growth performance and on their health condition, when included in the broiler chickens nutrition. In order to establish the effect on the growth performance, the following aspects were monitored during the experiment: changes in body weight, weight gain, feed intake, feed conversion ratio and flock losses. For the chickens' health condition evaluation, at the age of 40 days, from their blood the total antioxidant status and the haematological profile were determined and histological analyses were made on tissue fragments from the stomach (glandular, mechanical), the intestine (duodenum, cecum), the heart, the liver, the spleen, the kidneys, sampled after the chickens' sacrifice.

The fourth objective was to establish the effect of whole grape pomace and grape seeds used in the laying hen nutrition on the egg's productive and qualitative parameters. Thereby, in order to achieve this objective, during the experiment, the following productive parameters were monitored: weekly average and total feed consumption, egg production and feed *conversion* for egg production. The quality of the fresh eggs harvested the first day and after 30 days of winery by-products administration in the hens' nutrition was evaluated. For the evaluation of egg freshness degradation degree during storage, the eggs were stored in the fridge, at a 4°C temperature, and at the end of every storage period: 7, 14, 21, 28 days, the physical parameters of egg quality were determined; the same workflow was followed in the second month of the experiment as well.

The physical parameters of egg quality determined for the fresh eggs are as follows: eggs' and egg components' weight, shell thickness, yolk colour, egg freshness appreciated based on the value of the Haugh unit, specific gravity (using the saline solution method) and freshness appreciation points (yolk and albumen index).

The internal physical indicators of quality evaluated during the eggs storage at 4°C were the following: egg weight, specific gravity, Haugh unit, yolk and albumen index.

Also, at the end of the experiment, fresh eggs samples were harvested for the total antioxidant status determination (TAS) in the egg components (albumen, yolk), cholesterol and fatty acids in the yolk, and blood samples too, for the hens' blood TAS determination.

The experimental research was conducted in the biobase of the Faculty of Animal Breeding from the „Ion Ionescu de la Brad” USAMV, of Iași.

In the experiment carried out on the broiler chickens, from the meat hybrid *Cobb 500*, 250 hatched chickens (divided into six groups) were used, between the ages of 1 and 40 days, while

in the experiment carried out on the laying hens from the hybrid *Tetra-SL LL*, 48 hens (divided into four groups) with the age of 60 weeks were used.

The primary data resulted from the conducted research was processed using biostatistical methods. The following data processing methods were used: calculation the position and variation estimators, variation analysis, calculating the Pearson correlation coefficients, determining the simple regression equations (regression analysis), the „Boxplot” diagram (that highlights the values’ distribution).

In order to test the environments’ homogeneity and the significance of the differences between the environments the following calculation mathematical models were used: the ANOVA technique (variation analysis), the F test (the Fisher test for dispersions comparison) – based on the ANOVA technique, the t test (Student). The statistical data processing was automatically performed by a computer, using the Microsoft Office 2010 and 2016 (Microsoft Inc., SUA) software pack.

From the data generated by our own research on the preservation procedure and the chemical and nutritional description of some winery by-products and the possibilities of their use in the nutrition of some poultry categories, together with highlighting their influence on the obtained production’ s performance and quality, a series of conclusions were drawn, summarized as it follows:

- *Conclusions regarding the preservation procedure and the chemical content description:*

- a raise in the drying temperature (20°C, 38°C and 50 °C) causes a dramatic degradation on the amount of total polyphenols, tannins and anthocyanins contained by the grape pomace ($p < 0.001$); the optimal drying temperature for grape pomace is 20 °C (natural drying), in order to maintain the biologically active compounds to be used in the animal feeding.

- the collected data regarding the chemical composition of the four whole grape pomace types and their fractions, analyzed depending on the grape variety and on the obtaining year (2013, 2014) indicated the grape pomace from the Fetească neagră red grape variety, obtained in 2013-2014, as having a higher level of nutrients (PB%, GB%, SEN%, PT%, Ta%).

- a higher amount of total polyphenols (4.00% of DM) was detected in the grape pomace from the Fetească neagră variety, obtained in 2014, while the amount detected in the other analyzed grape pomace types was only between 1.90% (mixture) – 2.20% of DM (the Fetească neagră variety, 2013).

- taking into consideration the chemical content of the analyzed grape pomace's fractions, the research revealed a higher level of crude protein (12.13-19.31%), crude ashes (7.40-11.90%) and nitrogen-free extractive substances (49.64-54.67%) in the skins compared with the seeds. The seeds presented a higher level of crude fat (12.15-14.82%), crude cellulose (38.19-45.57%), total polyphenols (2.72-5.23%) and tannins (2.26-4.47%) than the skins.

- it is worth noting that the whole grape pomace showed a lower level of total polyphenols, between 1.90-4.00%, compared with the level in the seeds, with values between 2.72-5.23%.

- the fatty acids from the seeds of the Fetească neagră (2014) variety presented a higher level of polyunsaturated fatty acids (69.59%), than the level found in the whole grape pomace (61.08%). The seeds presented a higher level of linoleic fatty acid 69.14%, compared to whole grape pomace (59.73%).

- since a greater amount of chemical components, especially polyphenols, was detected in the grape pomace from the Fetească neagră red grape variety (2014), this was considered the most appropriate grape pomace to be used in the poultry nutrition.

- *Conclusions regarding the productive performance of the broiler chickens after including certain winery by-products in their nutrition:*

- from the presented data regarding the effect of grape pomace, seeds and polyphenols extract, it was concluded that the proportion of 0.5% seeds (LS-0.5%) used in the broiler chickens' nutrition improved the productive performance throughout the growth period. At the age of 40 days, the registered medium body weight was significantly higher ($p < 0.01$) and the feed conversion coefficient was 3.97% higher than the control lot.

- instead, the use of a higher proportion of the seeds in the nutrition (1%) affected the growth performance, with the possibility of a late effect on the chickens' growth. Throughout the growth period, the chickens have recorded average body weight and reduced food consumption, unlike the control group.

- the use of grape pomace in a proportion of 1 and 2% in the broiler chickens' nutrition had a slight positive influence on growth; the medium body weights were higher with 1.20 (at a 1% proportion) – 2.74% (at a 2% proportion) compared with the control group. The feed conversion coefficient registered values 2.66% higher (at a 1% proportion) than the control group.

- including the polyphenols extract in the chicken's (1.5% LEP group) drinking water affected the growth performance from the start of the experiment, the lower weight gain could be a consequence of the high level of polyphenols found in the grape pomace extract, with a possible late effect on the chickens' growth.

- the antioxidant activity and the investigated red series haematological indices did not highlight significant changes in the chickens from the experimental groups, with winery by-products included in their feed. Peripheral blood leukocytes presented changes in all the experimental groups, compared to the control group, causing a regulation of immune response (lymphocytes growth – $p>0.05$ at a 1% proportion, or weightlifters reduction – $p<0.05$ at a 2% proportion), or a hyperimmune reactivity (increased eosinophils and basophils in the blood, increase of IgA plasma cells in the intestinal villi's *lamina propria* – $p<0.001$) at the LEP, 2% proportion and 1% proportion groups.

- the duodenal villi's height increase was very significant at the 2% proportion - LT-2% group ($p<0.001$) and significant ($p<0.05$) at the chickens from the LT-1%, LS-1% and LEP groups, compared with the control group. The increase in the relation villi height/crypt depth was associated with an improvement in the growth performance for the LT-2% and LS-0.5% groups.

- the histological examination did not reveal important morphological changes in the internal organs for the experimental groups, which generally presented a normal aspect.

- depending on the winery by-products' quantity and administration form in the poultry nutrition, our results prove the polyphenols' contribution to the immune response modulation, both locally and systemically, and while small doses generate transmigration, differentiation and controlled proliferation of the immune system's cells, big doses induce hyperimmune phenomena and an increased development of intestinal villi.

- considering the above, winery by-products could be used as growth stimulators in the broiler chickens' nutrition, with the possibility of favouring the intestine's proper functioning, increasing the surface of nutrient absorption and inducing local and systemic immunomodulatory effects (depending on the dosage).

• *Conclusions regarding egg's productive and qualitative parameters of the laying hens after including certain winery by-products in their nutrition:*

- the presented data regarding the grape pomace and grape seeds' effect on the production performance shows that a proportion of 2 % seeds included in the laying hens' nutrition improved the egg production (with 2.80%), laying intensity (87.50% vs. 85.12%) and feed efficiency (0.43% lower IC).

- the use of grape pomace in proportions of 2 and 4% in the laying hens' nutrition had a negative impact, with significant differences in the productive performance; the decreased egg production (with 10.49% at the LT-2% group and with 6.76% at the LT-4% group) and the increased feed consumption (with 4.36% at the LT-2% group and with 4.04% at the LT-4%

group) determined a decreased feed efficiency (IC with 17.87% at the LT-2% group and with 11.91% at the LT-4% group).

- the presented data regarding the effect of grape pomace and grape seeds on the fresh eggs quality revealed that after 30 days the experimental factors did not have a significant influence ($p>0.05$) on the eggs' weight and components, specific weight, shell thickness or yolk colour. After 30 days, a significant improvement of the Haugh unit, albumen and yolk index was observed at the eggs from the LT-2% and LS-2% groups. The shell quality was not improved by the experimental factors, the shell thickness and weight decreased especially at the LT-2% group. The LS-2% group, however, recorded a higher level of shell weight compared to the level from the beginning of the experiment.

- the presented data regarding the effect of grape pomace and grape seeds on the biochemical indicators of the fresh eggs showed that the experimental factors used in the laying hens' nutrition positively influenced the fatty acids' composition in the fresh egg yolk. A proportion of 2% seeds used in the hens' nutrition (LS- 2%) improved the $\Omega 6/\Omega 3$ balance in the yolk (8.68 vs 9.43) through a significant increase in n-6 fatty acids amount [the eicosatrienoic acid - (C20:3n-6); the arachidonic acid (C20:4n-6)] and n-3 fatty acids [the eicosatrienoic acid (C20:3n-3) and the docosahexaenoic acid (C22:6n-3)]. Also, an improvement in the $\Omega 6/\Omega 3$ balance was noticed at the lot that received a proportion of 2 % grape pomace in the nutrition (8.96 vs.9.43), but no significant increase of the n-3 and n-6 fatty acids in the yolk was detected, except for the eicosatrienoic acid (C20:3n-3). The linoleic acid amount in the yolk did not present notable changes at the experimental lots; a higher level was detected at the lot that received a proportion of 4 % grape pomace in the nutrition (22.97% vs. 20.47%).

- the cholesterol amount in the yolk was not altered by the experimental factors, the values were similar to those of the control group.

- the experimental factors did not alter the total antioxidant status from the egg components and from the laying hens' serum, the registered values being similar to those of the control group. A higher level of the TAS in the serum was registered at the LS- 2% group (1.51 mmol/L vs. 1.44 mmol/L).

The data regarding the evaluation of egg freshness degradation degree during storage indicated that a proportion of 2 % seeds included in the laying hens' nutrition improved the egg's internal physical indicators throughout the storage period, with reduced decrease of the specific gravity (1.84%), yolk index (8.75%), Haugh unit (11.65%) and albumen index (9.69%). Significant decreases in the albumen index and Haugh unit were noticed in the third

week of storage ($p<0.001$) and significant decreases in the yolk index were noticed in the fourth week of storage ($p<0.05$) at the LS-2% group. This led to greater stability of the egg's internal characteristics during storage (28 days). The eggs from the LS-2% lot did not register significant differences regarding the indicators compared to the control lot throughout the storage period.

However, proportions of 2 and 4 % grape pomace included in the nutrition did not improve the physical indicators of quality during the storage period; a proportion of 2% grape pomace visibly affected the eggs' internal physical parameters, recording bigger decreases in the specific gravity (1.94%), albumen index (35.74%), Haugh unit (16.74%) and yolk index (11.69%) throughout the storage period. Significant decreases ($p<0.001$) of these parameters (Gs, Iy, Hu) were noticed at the LT-1% group from the first week of storage. After a 21 days storage, the eggs from the LT-2% and LT-4% groups showed significant decreases of the Haugh unit ($p<0.01$) compared to the control group. In the same storage period, the LT-2% group also registered a significant bigger decrease ($p<0.01$) of the albumen index compared to the control group.

The conducted research highlighted that the used winery by-products are of interest for the poultry nutrition, due to the nutrients amount, especially polyphenols and polyunsaturated fatty acids, and the experimental results confirmed that, depending on the proportion used in the nutrition, they can also have positive effects on the performance and health condition, as well as on some quality aspects of the obtained production.